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IS 6852 (1973, Reaffirmed 2000): Specification for Bile Salts, Microbiological Grade. UDC 547.931 : 576.8



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IS : 6852 - 1973

Indian Standard
SPECIFICATION FOR
BILE SALTS, MICROBIOLOGICAL GRADE

UDC 547.931 : 576.8



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INDIAN STANDARDS INSTITUTION
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110001

Price Rs 2.50

Gr 2

July 1973

Indian Standard

SPECIFICATION FOR BILE SALTS, MICROBIOLOGICAL GRADE

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Indian Standard

SPECIFICATION FOR BILE SALTS, MICROBIOLOGICAL GRADE

0. FOREWORD

0.1 This Indian Standard was adopted by the Indian Standards Institution on 28 February 1973, after the draft finalized by the Food Hygiene, Sampling and Analysis Sectional Committee had been approved by the Agricultural and Food Products Division Council.

0.2 Unless the ingredients used in media for microbiological work are of uniform quality, the results obtained might be erroneous and might be unreliable. Since the media used in different laboratories often differ greatly in their quality, the results of microbiological work at different laboratories cannot be compared. Therefore, with a view to unifying the practices of different laboratories dealing with microbiology and providing guidance to the indigenous manufacturers regarding the quality, it was decided to bring out a series of Indian Standard specifications for ingredients commonly used in media for microbiological work.

0.2.1 For specific purposes, any additional requirements shall be as agreed to between the purchaser and the supplier.

0.3 Bile salts are usually prepared from fresh ox-bile and besides other bile acids, contain a mixture of sodium glycocholate and sodium taurocholate. Bile salts are used principally in the preparation of selective media for the isolation and enumeration of the 'Coliform' bacteria.

0.4 For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test, shall be rounded off in accordance with IS:2-1960*. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

1. SCOPE

1.1 This standard prescribes the requirements and the methods of test for bile salts, microbiological grade.

*Rules for rounding off numerical values (*revised*).

2. REQUIREMENTS

2.1 In a medium containing 0.1 percent bile salts and pH adjusted to 7.5, there shall be inhibition of Gram-positive bacteria when tested according to the method prescribed in Appendix A.

2.2 In such media as MacConkey broth or MacConkey agar, there shall be no inhibition of Gram-negative bacteria, especially of those belonging to the family *Enterobacteriaceae*, when tested according to the method given in Appendix A.

2.3 In media containing 10 and 40 percent bile salts, there shall be growth of *Streptococcus faecalis*, when tested in accordance with the method given in Appendix A.

2.4 There shall be a growth of *Streptococcus hominis* in media containing 10 percent bile salts and no growth of this organism in media containing 40 percent bile salts, when tested according to the method given in Appendix A.

2.5 There shall be no growth of *Streptococcus dysgalactiae* in media containing 10 percent bile salts, when tested according to the method given in Appendix A (see A-2.2).

3. PACKING, STORAGE AND MARKING

3.1 **Packing** — The material shall be securely packed in well-filled wide mouth containers with tightly fitting lids.

3.2 **Storage** — The material shall be stored in a cool and dry place.

3.3 **Marking** — Each container shall be marked legibly to give the following informations:

- a) Name of the material including the words 'Microbiological grade'.
- b) Name and address of the manufacturer,
- c) Minimum net content, and
- d) Batch or code number.

3.3.1 The container may also be marked with the ISI Certification Mark.

NOTE — The use of the ISI Certification Mark is governed by the provisions of the Indian Standards Institution (Certification Marks) Act and the Rules and Regulations made thereunder. The ISI Mark on products covered by an Indian Standard conveys the assurance that they have been produced to comply with the requirements of that standard under a well-defined system of inspection, testing and quality control which is devised and supervised by ISI and operated by the producer. ISI marked products are also continuously checked by ISI for conformity to that standard as a further safeguard. Details of conditions, under which a licence for the use of the ISI Certification Mark may be granted to manufacturers or processors, may be obtained from the Indian Standards Institution.

4. SAMPLING

4.1 The representative samples of the material shall be drawn according to the method prescribed in 3 of IS : 6854-1973*.

5. TESTS

5.1 Tests shall be carried out by the methods prescribed in 2.

5.2 **Quality of Reagents** — Unless specified otherwise, pure chemicals and distilled water (see IS : 1070-1960†) shall be employed in tests.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the experimental results.

APPENDIX A

(Clauses 2.1 to 2.5)

TESTS FOR REQUIREMENTS OF BILE SALTS, MICROBIOLOGICAL GRADE

A-1. MEDIA

A-1.1 Mix and dissolve by heating 10.0 g of peptone, 10.0 g of meat extract, and 5.0 g of sodium chloride in 1 000 ml distilled water. Cool and adjust to pH 7.5. Remove precipitate by filtering through a filter paper. Use this base medium to prepare three sets of media, one containing 0.1 percent bile salts under test, second containing 10.0 percent bile salts under test and the third containing 40.0 percent bile salts under test. Add agar microbiological grade (see IS : 6850-1973‡) in such concentration as will solidify the medium and produce a sufficiently firm surface when poured in petri dishes. Heat to dissolve the agar and sterilize by autoclaving at 121°C for 15 minutes. Pour into sterile petri dishes.

A-2. INHIBITION OF GRAM POSITIVE BACTERIA

A-2.1 Inoculate 3 plates of the medium containing 0.1 percent bile salts with an overnight nutrient broth culture of *Staphylococcus aureus* and incubate at 37°C for 48 hours. There shall be no growth of the organism on any of the 3 plates.

A-2.2 Inoculate 3 plates of the medium containing 10.0 percent bile salts with an overnight nutrient broth culture of *Streptococcus dysgalactiae* and incubate at 37°C for 48 hours. There shall be no growth of the organism on any of the 3 plates.

*Methods of sampling and test for ingredients used in media for microbiological work.

†Specification for water, distilled quality (revised).

‡Specification for agar, microbiological grade.

A-3. NO INHIBITION OF GRAM NEGATIVE BACTERIA

A-3.1 MacConkey Agar Medium — Dissolve by heating 5.0 g of the bile salt under test, 20.0 g of peptone and 5.0 g of sodium chloride in 1 000 ml distilled water. Cool and adjust to pH 8.0, boil for 20 minutes, cool and filter. Add 20.0 to 30.0 g agar, microbiological grade (see IS: 6850-1973*), dissolve by boiling and adjust to pH 7.4. Add 10.0 g of lactose and 10.0 ml of 1.0 percent aqueous solution of neutral red. Mix and sterilize at 115°C for 20 minutes. More of neutral red may be added, if a deeper colour is required. For use, melt in a steamer and pour into sterile petri dishes.

A-3.2 To test the ability to support growth of strains of the family *enterobacteriaceae*, use representative cultures, such as of *Escherichia coli*, *Salmonella typhi*, *Shigella* sp. The growth on MacConkey agar with the bile salt under test should be as good as the growth of these organisms tested on nutrient agar (**A-1.1** but without bile salts). This is tested by simultaneously inoculating the cultures on the media, incubating at 37°C for 48 hours and observing the growth.

A-4. GROWTH OF *STREPTOCOCCUS FAECALIS* AND *STREPTOCOCCUS HOMINIS*

A-4.1 Inoculate overnight nutrient broth cultures each of *Streptococcus faecalis* and *Streptococcus hominis* separately on to 3 plates each, of media containing 10.0 percent and 40.0 percent of the bile salts under test. Incubate at 37°C for 48 hours. There shall be growth of *Streptococcus faecalis* on medium containing 10.0 percent as also containing 40.0 percent bile salts in all the plates. There shall be growth of *Streptococcus hominis* on medium containing 10.0 percent bile salts on all 3 plates and no growth of this organism in medium containing 40.0 percent bile salts on all 3 such plates.

*Specification for agar, microbiological grade.